Tropical Medicine and International Health

VOLUME 6 NO 12 PP 1008–1015 DECEMBER 2001

Review: Somatostatin and intestinal schistosomiasis: therapeutic and neuropathological implications in host–parasite interactions

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Summary A better insight into the mechanisms regulating the human body can lead to improved knowledge of the patho-physiological processes of many diseases. New therapeutic possibilities can be devised at the level of these regulatory mechanisms. Somatostatin is one of the major regulatory hormones in the central nervous system (CNS) and digestive system. Its wide variety of activities means it is implicated in a broad range of conditions. One symptom common to both the acute and chronic stages of schistosomiasis is intestinal pathology characterized by abdominal pain, diarrhoea that is bloody in more chronic stages, nausea and fever. Some chronic patients develop severe hepatosplenic fibrosis, leading to fatal oesophageal variceal bleeding. In this review we assess the therapeutic potential of somatostatin in the treatment of intestinal pathology associated with schistosomiasis. The activity of somatostatin is mediated via binding to specific cell surface receptors. While we are making progress in studies of the expression and regulation of the different somatostatin receptors, the true role and distribution of each receptor subtype is far from fully understood. Animal models will help to define the specific role of individual receptors in physiological and pathological conditions. The regulation of receptor expression as well as receptor internalization can give us insight into the effect of exogenous somatostatin on schistosomiasis-mediated intestinal pathology, as well as its modulation by intrinsically produced somatostatin levels.

keywords receptor, schistosomiasis, somatostatin, therapy

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Somatostatin

Pharmacology

As early as 1968, Kruhlich *et al.* postulated that the release of growth hormone (GH) by the anterior lobe of the pituitary gland is regulated by two opposing neurohormones, one that stimulates the release of GH (the growth hormone releasing factor or GRF), and another that inhibits it (Kruhlich *et al.* 1968). The inhibiting neurohormone was isolated in 1972 from sheep hypothalamus by Guillemin. The substance, a cyclic tetradecapeptide, was named somatostatin. A second native molecular form, somatostatin-28, was reported in 1980 (Pradyrol *et al.* 1980). Somatostatin-28 contains the amino acid sequence of somatostatin-14 with the extension of 14 amino acid residues at the N-terminal end. Pharmacological studies in humans have revealed the following characteristics.

Somatostatin is a natural and ubiquitous neurohormone, secreted by the central nervous system (CNS), the salivary glands, the stomach, the pancreas, the intestine and the urinary tract (Bloom & Polak 1987). From a quantitative and pharmacological point of view, the two major sites of secretion appear to be the CNS and the digestive tract (Reichlin 1983a,b; Polak & Bloom 1986). In the intestinal nervous system, duodenum and the pancreas, somatostatin-14 is the predominant molecular form, whereas the endocrine-type D cells of the intestine and the body of the stomach primarily contain somatostatin-28 (Green *et al.* 1989). Somatostatin exerts very specific activity depending upon its location. Its pharmacological specificity has led

researchers and clinicians to develop somatostatin in well-defined indications (Holt & McGregor 1996). The targets of somatostatin at various sites in the body depend on the presence and density of specific receptors (Patel 1999). Pharmacokinetic studies in animals and humans show its half-life to be about 2 min (Ho *et al.* 1986). Upon infusion into humans, a plateau level is reached within 15 min. Plateau levels depend upon the rate of infusion and vary between 300 and 3000 pg/ml at the usual therapeutic rate of 250 μg/h. The metabolic clearance rate is about 2000 ml/min. Plasma levels decrease rapidly on cessation of infusion. Chronic liver disease does not appear to modify this metabolic clearance of somatostatin.

Acute toxicity studies in animals show somatostatin to be very safe. Its LD50 in mice is about 10 000 times the acute therapeutic dose in humans. Chronic administration shows no particular untoward effect of somatostatin (D'Amico *et al.* 1995).

Mode of action

The degree of activity of somatostatin depends upon the concentration and affinity of specific receptors. High-affinity binding sites were identified in the CNS and digestive tract. The density and affinity of the receptors diminish along the gastrointestinal (GI) tract. In the lower intestine, the activity of somatostatin is virtually undetectable. Somatostatin is thought to act by the following means (Patel 1999): via activation of the G protein-dependent inhibitory subunit of adenylate cyclase; via activation of tyrosine phosphatases, or by reducing the influx of calcium into the cells.

Somatostatin in the human digestive system

In the digestive tract, somatostatin is variably distributed along the GI tract, and is secreted by the D cells of the gastro-entero-pancreatic (GEP) endocrine system (Bordi *et al.* 1996). These secretions are concentrated in the pancreas, gastric fundus and antrum, and the upper part of the small intestine. In the digestive tract, somatostatin acts either as a local paracrine or as a distant endocrine hormone.

Natural somatostatin inhibits a wide variety of physiologic regulatory functions in the GI tract, as well as in the pancreatic exocrine and endocrine secretions (Reichlin 1983a,b). Somatostatin is viewed as potentially useful in patients with a number of GI and pancreatic diseases. It prolongs the GI transit time, decreases endogenous fluid secretion in the jejunum, and stimulates intestinal absorption of water and electrolytes.

In pharmacological application, its inhibitory activity particularly affects biliary, pancreatic and GI secretions (Marteu *et al.* 1989; Anderson *et al.* 1996), splanchnic blood flow, and gastric motility. Somatostatin inhibits gastric acid secretion in a dose-dependent manner (Arnold *et al.* 1975). In normal subjects and patients with hypergastrinaemia, it lowers serum gastrin values (Schudsziarra 1996). Also, expected increases in plasma secretin levels induced by duodenal acidification did not occur in the five volunteers infused with 500 μg/h of somatostatin (Hanssen *et al.* 1977). Moreover, plasma motilin levels were reduced by 20% in four healthy volunteers receiving increasing doses of somatostatin (Peeters *et al.* 1981). Numerous studies in insulin-dependent diabetes and healthy volunteers showed a significant decrease in insulin and glucagon plasma levels during infusion of therapeutic doses of somatostatin (Schudsziarra 1996). Somatostatin induces a marked decrease in bile flow and bile acid secretion with an increase in bile cholesterol saturation (Marteu *et al.* 1989).

Somatostatin in therapeutic doses reduces splanchnic blood flow (Holt & McGregor 1996). Clements *et al.* (1986) demonstrated an additional lowering action of somatostatin on variceal pressure in portal hypertension. The reduction of splanchnic blood flow resulted in a significant lowering of intestinal absorbtion (Wahren & Felig 1976).

Increasing doses of somatostatin infused into eight healthy volunteers caused complete inhibition of gastric motility, even at the lowest dose of 1.2 pM/kg/min for 90 min (Peeters *et al.* 1981).

Somatostatin receptors

The action of somatostatin is mediated through specific receptors that are functionally coupled to inhibition of adenylate cyclase via G-proteins (Patel 1999). Five cell surface somatostatin receptors have been characterized, termed SSTR-1 to SSTR-5 according to the chronology of their discovery, and because they all display the structural hallmark of the seven transmembrane domain receptor (SSTR is the acronym for Somatostatin Seven Transmembrane domain Receptor).

Human SSTRs (hSSTRs) are encoded by a family of five genes which map to separate chromosomes, and which with one exception are intronless. SSTR-2 gives rise to spliced variants, SSTR-2A and 2B. hSSTR-1 and 4 display selectivity for SST-14 binding. The larger molecular form (somatostatin-28) has a greater binding affinity than somatostatin-14 for SSTR-5. Octreotide, a somatostatin analogue currently used, has high binding affinity for the SSTR-2 and SSTR-5 receptor subtypes. However, octreotide has only an intermediate binding affinity for SSTR-3 (somatostatin-14) and has little or no affinity for SSTR-1

and SSTR-4 subtypes (Panetta *et al.* 1994). The SSTR subtypes present a high degree of sequence identity (39–57%). The sequence differences reside in the extracellular and intracellular domains and are probably responsible for their signalling specificity. Each receptor subtype is coupled to multiple intracellular transduction pathways via the G-proteins, and all five SSTRs are functionally coupled to inhibition of adenylate cyclase (Patel & Shrikant 1994; Patel *et al.* 1995).

In neuronal cells somatostatin regulates several subsets of K^+ channels causing hyperpolarization of the plasma membrane and leading to decreased Ca^{2+} influx through voltage-gated Ca^{2+} channels, and consequently to a reduction of intracellular Ca^{2+}. SSTR3 is involved in the effect of somatostatin on guinea-pig intestinal smooth muscle cell contraction mediated by phospholipase C. In the stomach SSTR2 contributes to the inhibition of histamine and gastrin release and to the inhibition of gastric acid secretion. SSTR1 and 2 mediate the inhibition of intestinal ionic secretion. SSTR2 and 5 may be involved in the inhibition of Ca^{2+} influx and thereby inhibition of neurotransmitters and hormones. SSTR3 could be involved in gastric and intestinal smooth muscle contraction and SSTR5 in the inhibition of colonic muscle cell contraction (Benali *et al.* 2000).

In the human GI tract three types of tissues express somatostatin receptors: (1) the GI mucosa, (2) the peripheral nervous system, and (3) the gut-associated lymphoid tissue, where the receptors are preferentially located in germinal centres. Somatostatin receptors are also expressed in pathological states, particularly in neuroendocrine tumours of the GI tract (Reubi 1992). However as observed for other G protein-coupled receptors, somatostatin receptors are sensitive to internalization. SSTRs1–3 are internalized in response to somatostatin, whereas SSTR4 is not. Phosphorylation of the serine/threonine residues at the C-terminal domain of the SSTRs plays an important role in the SSTR internalization process (Meyerhof 1998).

Intestinal schistosomiasis

Schistosomiasis is a parasitic disease caused by infection with trematodes belonging to the genus *Schistosoma*. *Schistosoma mansoni* infections are prevalent in Africa and South America. The occurrence of this infection depends on warm climates, the presence of, and a suitable environmental habitat for, a snail intermediate host of the genus *Biomphalaria*, insufficient sanitary conditions allowing faecal water contamination and human water contact with infected water bodies (WHO Expert Committee 1993).

Adult *S. mansoni* worms live in the mesenteric veins around the human intestines as a couple, the male surrounding the female in a gynaecophoric canal. The adult female worm produces between 300 and 3000 eggs per day. The eggs penetrate the tissues actively by means of histolytic enzymes and lodge themselves within the gut wall. Egg antigens secreted into the surrounding tissue trigger granulomatous, inflammatory responses consisting of macrophages, eosinophils and lymphocytes. This inflammation is responsible for the intestinal pathology associated with this disease. *Schistosoma mansoni* causes both acute and chronic disease with a wide range of symptoms. In the acute phase intestinal manifestations like abdominal pain, disturbed GI contractility leading to diarrhoea, nausea and fever occur. The main impact on public health is due to the chronic infection leading to severe intestinal involvement resulting in bloody diarrhoea. Besides this there is hepatosplenic involvement where hepatic fibrosis can lead to portal hypertension and oesophageal varices, which in some cases is complicated with oesophageal bleeding that may be fatal. But only a small proportion of infected patients develop severe and terminal schistosomiasis. Therefore it is the intestinal involvement of *S. mansoni* infection that is most important in terms of public health (Gryseels 1992).

Ileal muscle hyperreactivity in *S. mansoni* infections

Schistosoma mansoni egg-caused inflammatory reactions in the gut epithelium alter GI motility in humans and in experimental animal models (Gryseels 1992; Domingo & Warren 1969; Moreels *et al.* 2001). The mouse is a fully susceptible host to *S. mansoni* and can be used to study the effects of *S. mansoni* infection on GI muscle contractility. In acute as well as chronic *S. mansoni*-induced gut inflammation, both structural and functional changes occur in the ileum. These changes are characterized by increased ileal wall thickness and smooth muscle contractility restricted to the inflamed gut segment (Moreels *et al.* 2001). During the chronic phase of inflammation the ileal muscle contractility is increased irrespective of the contractile stimulus used, be it via the activation of muscarinic receptors on the muscle cells (triggered by acetylcholine), or via activation of the enteric nerves [triggered by electrical field stimulation (EFS)].

It is speculated that the effect of *S. mansoni* infection on the smooth muscle function might be indirect through alterations of the myenteric plexus, but both morphological and functional alterations of the longitudinal smooth muscle layer lead to GI motor dysfunction in the infected host. The role of the myenteric plexus in the schistosomiasis-induced alterations of GI motility remains an enigma.

As the increased muscle contractility does not depend on the type of the contractile stimulus, it is possible that this overall hypercontractility disturbs the integrated propulsive activity of the normal gut, leading to a dysfunction of peristalsis and GI transit (Domingo & Warren 1969).

Neuronal dysfunction

Schistosoma-mediated inflammation of the GI tract involves the enteric nervous system. Morphological alterations of the myenteric plexus have been described (Varilek *et al.* 1991), and myenteric plexus destruction is said to lead to an increase in intestinal muscle thickness (Hadzijahic *et al.* 1993). Enteric nerves may play a role in the initiation and maintenance of intestinal inflammation but in a later-stage inflammation may also alter the normal function of enteric nerves (Aubé *et al.* 1996). Patients with inflammatory bowel disease show nerve fibre hypertrophy and architectural alterations in the nerve plexuses and nerve cell bodies in the enteric nervous system. Animal studies demonstrated alterations of sympathetic and parasympathetic nerve function during acute jejunal inflammation (Collins *et al.* 1989) and during chronic intestinal inflammation (Venkova *et al.* 2000), suggesting that inflammation of the GI tract may lead to a functional reorganization of intrinsic and extrinsic neuronal function. The underlying mechanisms of such a reorganization remain to be elucidated.

In the GI tract, acetylcholine is the primary neural regulator of GI motility, and modulation of cholinergic nerve activity directly affects intestinal smooth muscle contractility (Burks 1994). It is known that cholinergic nerves in the enteric nervous system contain a variety of pre-junctional receptors. Activation of these receptors may inhibit or facilitate the release of acetylcholine (Wood 1994). In normal physiological conditions this allows a fine tune of acetylcholine release. The pre-synaptic modulation of neurotransmitter release is an important mechanism that directly modulates intestinal motility. But in pathological conditions inflammatory mediators (e.g. histamine) may act on neuronal receptors and disrupt the normal modulation of enteric cholinergic nerve activity. Such interactions between inflammatory mediators and enteric cholinergic nerves may contribute to the motility disturbances that are observed during chronic inflammatory diseases of the intestine.

In chronically inflamed ileum of *S. mansoni* infected mice, there is a hyperreactivity of nicotinic receptors. Enhanced sensitivity of neuronal nicotinic receptors during schistosomiasis may directly alter normal motility patterns. Neurotransmitters modulate their own release by activation of autoreceptors that are located on the nerve terminals. In the intestine, nicotinic receptors are thought to be located on the cell stroma and dendrites of the neurones in the myenteric plexus (Töröcsik *et al.* 1991). There is, however, recent evidence that nicotinic receptors are also located at the nerve terminal (Galligan 1999), where they may be involved in presynaptic modulation of neurotransmitter release (Schneider *et al.* 2000). Therefore a hyper-reactivity of nicotinic receptors during chronic inflammation induced by schistosomiasis may result in an enhanced activity of cholinergic nerves and disturb normal GI motility.

Somatostatin and intestinal schistosomiasis

During acute as well as chronic intestinal schistosomiasis, pathological conditions involve disturbed intestinal motility and pain. In animal model studies this disturbed motility is found to occur due to hyperreactive GI muscle contractility. Based on the knowledge we have now on human physiology and the pathology of acute and chronic intestinal schistosomiasis, one is prompted to ask whether somatostatin is a possible therapeutic agent for the treatment of motility disturbances during schistosomiasis. This question can be addressed taking into consideration the nature and behaviour of somatostatin receptors in normal as compared with pathological conditions.

Somatostatin receptors in normal conditions

Takeda *et al.* (1989) observed that somatostatin induced both a stimulatory as well as an inhibitory effect on cholinergic neurones of the guinea-pig ileum. SSTR3 and SSTR5 receptors located on the cholinergic nerves (Feniuk *et al.* 1993) mediate this inhibition. However, somatostatin may also activate SSTR3 receptors on the intestinal smooth muscle cells (Murthy *et al.* 1996).

In an attempt to correlate the various effects of somatostatin in GI pathology to individual SSTR subtypes, mRNAs have been localized by semiquantitative RTPCR amplification and *in situ* hybridization of SSTR1 and 3 in the rat GI tract. SSTR1-4 mRNAs were found throughout the GI tract, SSTR1 mRNA being more abundant than SSTR2 and much more abundant than SSTR3 and SSTR4 mRNAs. SSTR5 transcripts were at the threshold level. SSTR1 and 3 mRNAs are present in enterocytes and enteric neurones, suggesting a role of these subtypes in the somatostatin-mediated inhibition of acetylcholine release from the myenteric neurones. The presence of SSTR3 mRNA in smooth muscle cells points to an additional role of this receptor in regulating gut motility (Schafer & Meyerhof 1999). In the human GI tract the immunohistochemical detection of SSTR2a receptors showed the pres-

ence of specific receptor protein in the GI lymphatic and nervous components, but not in the GI circular and longitudinal smooth muscle (Reubi *et al.* 1999).

Somatostatin receptors during schistosomiasis

During *Schistosoma* infection, somatostatin receptor SSTR2 is expressed by inflammatory cells and directly regulates T cell IFN-gamma release. Elliott *et al.* (1999) have located the source of somatostatin in *S. mansoni*-infected mice. The granulomas surrounding the parasite egg in the liver and intestinal epithelium produce somatostatin. The SSTR2 receptor subtype is expressed on the surface of inflammatory T cells; they specifically bind somatostatin which down regulates IFN-gamma production by these cells. This finding has created a basis to delineate the immunomodulatory role of somatostatin in schistosomiasis.

Therapeutic effect of somatostatin during schistosomiasis

In recent years somatostatin is reported to demonstrate therapeutic effects in *S. mansoni*-infected mice. Somatostatin induced a reduction in the degree of hepatic fibrosis, portal pressure, weight of the spleen and liver, the liver egg load and granuloma size and cellularity (Mansy *et al.* 1998). Addition of this neuropeptide also inhibits alpha smooth muscle actin production by activated hepatic stellate cells (Reynaert *et al.* 1999; unpublished data). These reports strengthen the feasibility of using somatostatin for therapeutic purposes in schistosomiasis.

In view of the somatostatin ligand–receptor interaction during schistosomiasis, the hypothesis has been generated that schistosomiasis and the associated inflammatory response could cause an up-regulation of somatostatin receptors in the GI tract. If this were so, exogenously administered somatostatin would reduce the muscular hyperreactivity associated with the inflammation, and thereby overcome GI motility disturbances during schistosomiasis. A lead in this direction could pave the way for the efficacious use of somatostatin for relief from disturbed GI motility and pain.

In this context, research is ongoing to study the effects of somatostatin on EFS-triggered GI muscle contractility in *S. mansoni*-infected mice, using organ bath experiments (J.G. De Man, unpublished data). The effect of somatostatin on EFS-triggered muscle contractility is, in fact, marked in normal mice; but in chronic *S. mansoni*-infected mice there is a loss of the inhibitory effect of somatostatin concentrations on EFS-mediated ileal muscle contractility. We know of the presence of somatostatin in the inflammatory cells of the granulomas in *S. mansoni*-infected mice

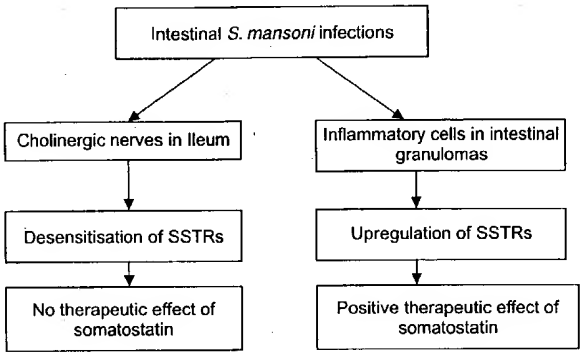

Figure 1 Intestinal *Schistosoma mansoni* infections may cause a desensitization of SSTRs on the cholinergic nerves of the inflamed ileum; however, lead to an upregulation of SSTRs on the inflammatory cells surrounding the intestinal granulomas. This implies no possible therapeutic effect of exogenously administered somatostatin on the cholinergic nerves but points to a positive therapeutic effect on the inflammatory cells.

(Elliott *et al.* 1999). This accumulation of endogenous somatostatin in and around the ileal mucosa and muscle layers may have led to receptor desensitization (Figure 1).

Target cells exposed to a ligand for a prolonged period of time often lose the ability to respond to that ligand (Kahn & Roth 1975; Raff 1976; Lefkowitz 1978). This adaptation or desensitization makes cells sensitive to changes in the concentration of a chemical signal rather than to the absolute concentration of the signal. Desensitization can occur from a decrease in the number of specific cell-surface receptor proteins or from the inactivation of such receptors; in other cases, it is due to changes in the proteins involved in transducing the signal following receptor activation.

In the case of somatostatin there is some evidence of target cell desensitization. Somatostatin receptor desensitization was reported to occur rapidly after addition of somatostatin analogues in the guinea-pig ileum (Feniuk *et al.* 1993). After somatostatin has bound to receptors on the surface of target cells, they are often ingested by receptor-mediated endocytosis. As endocytic vesicles generally deliver their contents to lysosomes, the ligand and often the receptor to which it is bound is degraded by hydrolytic enzymes. This process not only represents a major pathway for the breakdown of some signalling ligands, it also plays an important part in regulating the concentration of certain receptor proteins on the surface of target cells. Although receptor degradation and replacement takes place continuously, in the absence of the ligand a receptor usually has a half-life of a day or so. By inducing endocytosis, some ligands markedly increase the rate of receptor degradation so that at high ligand concentrations

the number of cell surface receptors gradually decreases. The result is a concomitant decrease in the sensitivity of the target cell to the ligand. This type of target cell desensitization is called receptor down-regulation (Kahn & Roth 1975; Raff 1976; Lefkowitz 1978).

Koenig & Edwardson (1997) have reported the endocytosis of somatostatin receptors. SSTR2A and SSTR3 internalize rapidly and efficiently (Nouel *et al.* 1997), whereas SSTR4 does not internalize (Kreienkamp *et al.* 1998), and a combination of recycling and recruitment of spare receptors protects SSTR5 from long-term down regulation (Stroh *et al.* 2000).

Conclusion

The exact mechanism whereby somatostatin loses its inhibitory effect on cholinergic nerve activity during *S. mansoni* infection remains to be investigated (Figure 1). The desensitization of somatostatin receptors on the cholinergic neurones could result in their diminished activity to exogenous somatostatin. This absence of effect of somato- statin on neurogenic function does not imply that exogenously added somatostatin would be ineffective on the inflammatory cells surrounding the granulomas. Somatostatin would prevent the degranulation of inflammatory mast cells, thereby preventing the release of histamine and other inflammatory mediators known to cause GI motility disturbances. As SSTRs are up-regulated on the inflammatory cells, somatostatin may inhibit the inflammatory response and (indirectly) normalize GI function. Exogenously added somatostatin will not further disturb GI motility as the SSTRs on the enteric nerves are apparently desensitized. This review suggests that somatostatin could be an effective therapeutic agent for intestinal schistosomiasis. This phenomenon may be specific for schistosomiasis and poses new debates in this field.

Acknowledgements

This work was supported by the Interuniversity Poles of Attraction Program (Grant P4/16, Services of the Prime Minister-Federal Agency for Scientific, Technical and Cultural Affairs).

References

Anderson HB, Petronijevic L, Giese B, Mygind T & Burcharth F (1996) Somatostatin reduces bile secretion and loss of bile constituents in patients with external biliary drainage. *HPB Surgery* 9, 229–233.

Arnold R, Kobberling J, Track NS & Creutzfeldt W (1975) Lowering of basal and stimulated serum immunoreactive gastrin and gastric secretion in patients with Zollinger–Ellison syndrome by somatostatin. *Acta Endocrinology* 193, S75.

Aubé AC, Blottiére HM, Scarpignato C, Cherbut C, Rozé C & Galmiche JP (1996) Inhibition of acetylcholine induced intestinal motility by IL1 beta in the rat. *Gut* 39, 470–474.

Benali N, Ferjoux G, Puente E, Buscail L & Susini C (2000) Somatostatin receptors. *Digestion* 62 (Suppl. 1), S27–S32.

Bloom SR & Polak JM (1987) Somatostatin. *British Medical Journal* 295, 288–290.

Bordi C, D'Adda T, Azzoni C & Ferraro G (1996) The somatostatin cell. In: *Octreotide, from Basic Science to Clinical Medicine* (ed. C Scarpignato) Karger, Basel, pp. 2–22.

Burks TM (1994) Neurotransmission and neurotransmitters. In: *Physiology of the Gastrointestinal Tract* (eds LR Johnson, DH Alpers, J Christensen, ED Jacobson & J Walsh) Raven Press, New York, pp. 211–242.

Clements D, Rhodes JM & Elias E (1986) Effect of somatostatin on oesophageal variceal pressure assessed by direct measurement. *Journal of Hepatology* 2, 262–266.

Collins SM, Blennerhassett PA, Blennerhassett MG & Vermillion DL (1989) Impaired acetylcholine release from the myenteric plexus of *Trichinella*-infected rats. *American Journal of Physiology* 257, G898–G903.

D'Amico G, Pagliaro L & Bosch J (1995) The treatment of portal hypertension: a meta-analytic review. *Hepatology* 22, 332–354.

Domingo EO & Warren KS (1969) Pathology and pathophysiology of the small intestine in murine *Schistosomiasis mansoni*, including a review of the literature. *Gastroenterology* 56, 231–240.

Elliott DE, Li J, Blum AM, Metwali A, Patel YC & Weinstock JV (1999) SSTR2A is the dominant somatostatin receptor subtype expressed by inflammatory cells, is widely expressed and directly regulates T cell IFN-gamma release. *European Journal of Immunology* 29, 2454–2463.

Feniuk W, Dimech J & Humphrey PP (1993) Characterization of somatostatin receptors in guinea pig isolated ileum, vas deferens and right atrium. *British Journal of Pharmacology* 110, 1156–1164.

Galligan JJ (1999) Nerve terminal nicotinic cholinergic receptors on excitatory motoneurons in the myenteric plexus of guinea pig intestine. *Journal of Pharmacological and Experimental Therapy* 291, 92–98.

Green DW, Gomez J & Greenley GH (1989) Gastrointestinal peptides. *Gastroenterology Clinics of North America (Philadelphia, PA)* 18, 695–733.

Gryseels B (1992) Morbidity due to infection with *Schistosoma mansoni*: an update. *Tropical and Geographical Medicine* 44, 189–200.

Hadzijahic N, Renehan WE, Ma CK, Zhang X & Fogel R (1993) Myenteric plexus destruction alters morphology of rat intestine. *Gastroenterology* 105, 1017–1028.

Hanssen LE, Hanssen KF & Myren J (1977) Inhibition of secretin release and pancreatic bicarbonate secretion by somatostatin

infusion in man. *Scandinavian Journal of Gastroenterology* 12, 391–394.

Ho LT, Chen RL, Chou TY, Fong JC, Wong PS & Chou CK (1986) Pharmacokinetics and effects of intravenous infusion of somatostatin in normal subjects – a two-compartment open-model. *Clinical and Physiological Biochemistry* 4, 257–267.

Holt RIG & McGregor AMM (1996) Somatostatin: from basic science to clinical applications. *Excerpta Medica Asia (Hong Kong)*, 1–146.

Kahn CR & Roth J (1975) Cell membrane receptors for polypeptide hormones: applications to the study of disease states in mice and men. *American Journal of Clinical Pathology* 63, 656–668.

Koenig JA & Edwardson JM (1997) Endocytosis and recycling of G protein-coupled receptors. *Trends in Pharmacological Science* 18, 276–287.

Kreienkamp HJ, Roth A & Richter D (1998) Rat somatostatin subtype 4 can be made sensitive to agonist-induced internalisation by mutation of a single threonine (residue 331). *DNA Cell Biology* 17, 869–878.

Kruhlich L, Dharival APS & McCann SM (1968) Stimulation and inhibitory effects of purified hypothalamic extracts in growth hormone release from rat pituitary *in vitro*. *Endocrinology* 83, 783–790.

Lefkowitz RJ (1978) Regulation of beta-adrenergic receptors by beta-adrenergic agonists. In: *Receptors and Hormone Action*, Vol. 3 (eds L Birnbaumer & BW O'Malley) Academic Press, New York, pp. 179–194.

Mansy SS, Yehia HA, Hassan MM, Hassan E, Youssef MM, Hadi AA & Mackenzie CD (1998) Effect of octreotide on the pathology of hepatic schistosomiasis. *Arzneimittelforschung* 48, 855–861.

Marteu P, Chretien Y, Calmus Y, Parc R & Poupon P (1989) Pharmacological effect of somatostatin on bile secretion in man. *Digestion* 42, 16–21.

Meyerhof W (1998) The elucidation of somatostatin receptor functions: a current view. *Reviews of Physiological and Biochemical Pharmacology* 133, 55–108.

Moreels TG, De Man JG, Bogers JJ *et al.* (2001) Effect of *Schistosoma mansoni*-induced granulomatous inflammation of murine gastrointestinal motility. *American Journal of Physiology* 280, G1030–G1042.

Murthy KS, Coy DH & Makhlouf GM (1996) Somatostatin receptor-mediated signalling in smooth muscle. Activation of phospholipase C-beta3 by G-betagamma and inhibition of adenylyl cyclase by Galphai1 and Galphao. *Journal of Biological Chemistry* 271, 23458–23463.

Nouel D, Gaudriault G, Houle M *et al.* (1997) Differential internalisation of somatostatin in COS-7 cells transfected with SST1 and SST2 receptor subtypes: a confocal microscopic study using novel fluorescent somatostatin derivatives. *Endocrinology* 138, 296–306.

Panetta R, Greenwood MT, Warszynska A *et al.* (1994) Molecular cloning, functional characterisation and chromosomal localisation of a novel human somatostatin receptor (SSTR5) with preferential affinity for somatostatin 28. *Molecular Pharmacy* 45, 417–427.

Patel YC (1999) Somatostatin and its receptor family. *Frontiers in Neuroendocrinology* 20, 157–198.

Patel YC, Greenwood MT, Panetta R, Demchyshyn L, Niznik H & Srikant CB (1995) The somatostatin receptor family. *Life Sciences* 57, 1249–1265.

Patel YC & Shrikant CB (1994) Subtype selectivity of peptide analogs for all five cloned human somatostatin receptors (hSSTR 1-5). *Endocrinology* 135, 2814–2817.

Peeters TL, Depraetere Y & Vantrappen GR (1981) Simple extraction method and radioimmunoassay for somatostatin in human plasma. *Clinical Chemistry* 27, 888–891.

Polak JM & Bloom SR (1986) Somatostatin localisation in tissues. *Scandinavian Journal of Gastroenterology* 21 (Suppl. 119), S11–S21.

Pradyrol L, Jornvuall H, Mutt V & Ribert A (1980) A N-terminally extended somatostatin: the primary structure of somatostatin 28. *FEBS Letters* 109, 55–58.

Raff M (1976) Self-regulation of membrane receptors. *Nature* 259, 255–266.

Reichlin S (1983a) Somatostatin. Part One. *New England Journal of Medicine* 309, 1495–1501.

Reichlin S (1983b) Somatostatin. Part Two. *New England Journal of Medicine* 309, 1556–1563.

Reubi JC (1992) Somatostatin receptors in the gastrointestinal tract in health and disease. *Yale Journal of Biology and Medicine* 65, 493–503.

Reubi JC, Laissue JA, Waser B, Steffen DL, Hipkin RW & Schonbrunn A (1999) Immunohistochemical detection of somatostatin sst2a receptors in the lymphatic, smooth muscular, and peripheral nervous systems of the human gastrointestinal tract: facts and artefacts. *Journal of Clinical and Endocrinological Metabolism* 84, 2942–2950.

Reynaert H, Vaeyens F, Qin H, Hellemans K, Chatterjee N, Winan D, Urbain D & Geerts A (1999) Activated rat hepatic stellate cells express somatostatin receptor subtypes 1, 2 and 3. *Hepatology* 30, 236A.

Schafer J & Meyerhof W (1999) sst1 mRNA is the prominent somatostatin receptor mRNA in the rat gastrointestinal tract: reverse transcription polymerase chain reaction and in situ-hybridization study. *Neuropeptides* 33, 457–463.

Schneider DA, Perrone M & Galligan JJ (2000) Nicotinic acetylcholine receptors at sites of neurotransmitter release to the guinea pig intestinal circular muscle. *Journal of Pharmacology and Experimental Therapy* 294, 363–369.

Schudsziarra V (1996) Somatostatin: biological actions and pathophysiology In: *Octreotide: From Basic Science to Clinical Medicine* (ed. C Scarpignato) Karger, Basel, pp. 35–53.

Stroh T, Jackson AC, Sarret P *et al.* (2000) Intracellular dynamics of sst5 receptors in transfected COS-7 cells: maintenance of cell surface receptors during ligand-induced endocytosis. *Endocrinology* 141, 354–365.

Takeda T, Taniyama K, Baba S & Tanaka C (1989) Putative mechanisms involved in excitatory and inhibitory effects of

somatostatin on intestinal motility. *American Journal of Physiology* 257, G532–G538.

Töröcsik A, Oberfrank F, Sershen H, Lajtha A, Nemesy K & Vizi ES (1991) Characterisation of somatodendritic neuronal nicotinic receptors located on the myenteric plexus. *European Journal of Pharmacology* 202, 297–302.

Varilek GW, Weinstock JV, Williams TH & Jew J (1991) Alterations of the intestinal inervation in mice infected with *Schistosoma mansoni*. *Journal of Parasitology* 77, 472–478.

Venkova K, Dunn ST, Adesina AM & Grenwood-van Meerveld B (2000) Neuromuscular dysfunction in the jejunum and colon of human leukocyte antigen B27 transgenic rats. *Journal of Pharmacology and Experimental Therapy* 293, 60–66.

Wahren J & Felig P (1976) Influence of somatostatin on carbohydrate disposal and absorption in diabetes mellitus. *Lancet* 2, 1213–1216.

WHO Expert Committee (1993) The control of schistosomiasis. *World Health Organisation Technical Report Series* 830, 1–86.

Wood JD (1994) Physiology of the enteric nervous system. In: *Physiology of the Gastrointestinal Tract* (eds LR Johnson, DH Alpers, J Christensen, ED Jacobson & J Walsh). Raven Press, New York, pp. 423–482.